

WHAT IS CLAIMED IS:

1. A method for determining the level of resistance of HIV to an HIV RT inhibitor

comprising:

a) providing a reaction well comprising

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor,

at least one ribonucleotide chosen from ATP and GTP or at least one

pyrophosphate;

b) adding to the reaction well an HIV RT enzyme chosen from a wild-type RT enzyme or a mutant RT enzyme, wherein said HIV RT enzyme incorporates the detectable dNTP substrate or the at least one HIV RT inhibitor into said template;

c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;

d) determining the level of resistance of HIV to the HIV RT inhibitor using the RT activity.

2. The method of claim 1, wherein the template is bound to the reaction well and is chosen from poly-rA or a heteropolymer RNA or DNA.

3. The method of claim 1, wherein the primer is chosen from oligo-dt or a primer that is complementary to the heteropolymer template.
4. The method of claim 1, wherein the detectable dNTP substrate is chosen from a radioactive labeled dNTP.
5. The method of claim 1, wherein the detectable dNTP substrate is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
6. The method of claim 1, wherein the detectable dNTP substrate binds to an optical tracer or a radioactive labeled tracer.
7. The method of claim 6, wherein the optical tracer is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
8. The method of claim 6, wherein the detectable dNTP precursor is bromo-deoxyuridine-triphosphate.
9. The method of claim 7, wherein the optical tracer is a monoclonal anti-BrdU antibody, conjugated to alkaline phosphatase.

10. The method of claim 1, wherein the HIV RT inhibitor is chosen from AZT, 3TC, ddI, ddC, d4T, and abacavir.
11. The method of claim 1, wherein the HIV RT inhibitor is chosen from a nucleoside or a nucleoside analog.
12. The method of claim 11, wherein the HIV RT inhibitor is a triphosphate form of the HIV RT inhibitor.
13. The method of claim 1, wherein the mutant RT enzyme contains mutations at codons 67, 69 and 70.
- ~~14. The method of claim 13, wherein the mutations at codon 69 are insertions.~~
15. A method for designing a therapy for treating HIV comprising:
determining the resistance of HIV to an HIV RT inhibitor using the method of claim 1;
designing the a therapy based on the resistance of HIV to the HIV RT inhibitor.
16. A method for designing a therapy for treating HIV comprising:
determining the resistance of HIV to an HIV RT inhibitor using the method of claim 1;

repeating the assay of claim 1, wherein the reaction well further contains a second HIV RT inhibitor;

determining the change in RT activity caused by the addition of the second HIV RT inhibitor; and

designing the therapy based on the change in RT activity due to the addition of the second HIV RT inhibitor.

17. A kit for determining resistance of HIV to an HIV RT inhibitor comprising

at least one template for an HIV RT enzyme;

at least one primer;

at least one detectable dNTP substrate; and

at least one ribonucleotide chosen from ATP and GTP or at least one pyrophosphate.

18. The kit according to claim 17, further comprising at least one mutant RT enzyme.

19. A method for determining the mechanism of action of an HIV RT inhibitor comprising:

a) providing a reaction well comprising

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor,

at least one ribonucleotide chosen from ATP and GTP or at least one pyrophosphate;

b) adding to the reaction well an HIV RT enzyme chosen from a wild-type RT enzyme or a mutant RT enzyme, wherein said HIV RT enzyme incorporates the detectable dNTP substrate or the at least one HIV RT inhibitor into said template;

c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;

d) determining the mechanism of action of the HIV RT inhibitor using the RT activity.

20. A method for determining the effect of at least one mutation in an HIV RT enzyme on the resistance of HIV to an HIV RT inhibitor comprising:

a) providing a reaction well comprising

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor, and

at least one ribonucleotide chosen from ATP or GTP or at least one pyrophosphate;

b) adding to the reaction well an HIV RT enzyme, wherein said HIV RT enzyme incorporates the at least one detectable dNTP substrate or the at least one HIV RT inhibitor into said template;

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- c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;
 - d) repeating steps a) through c) in a new reaction well wherein the HIV RT enzyme of step b) is chosen from at least one mutant RT enzyme;
 - e) comparing the RT activity in the different reaction wells; and
 - f) determining the effect of the at least one mutation on the resistance of HIV to an HIV RT inhibitor using step e.

21. A method for rapid screening the effects of mutations on HIV resistance to an HIV RT inhibitor comprising:

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- a) providing an array of reaction wells, each reaction well comprising:
 - at least one template for an HIV RT enzyme,
 - at least one primer,
 - at least one detectable dNTP substrate,
 - at least one HIV RT inhibitor, and
 - at least one ribonucleotide chosen from ATP or GTP or at least one pyrophosphate;
 - b) adding to each reaction well a different HIV RT enzyme chosen from a wild-type RT enzyme or a mutant RT enzyme, wherein said HIV RT enzyme incorporates the at least one detectable dNTP substrate or the at least one HIV RT inhibitor into said template;
 - c) determining RT activity in each reaction well by measuring the amount of the detectable dNTP substrate incorporated into the template; and

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d) determining the effect of mutations on HIV resistance to the HIV RT inhibitor using step c.

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